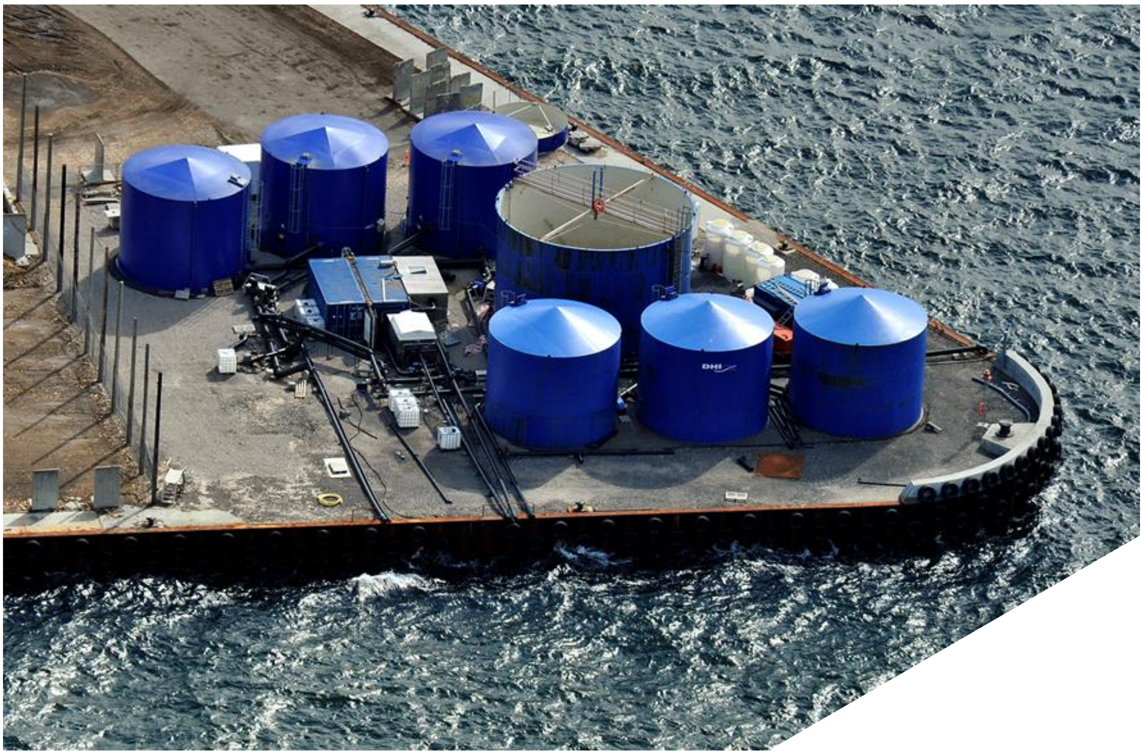
Whole effluent toxicity tests

RayClean

Filtration and UV disinfection



Desmi Ocean Guard A/S

Final Report

November 2014

The expert in **WATER ENVIRONMENTS**

This report has been prepared under the DHI Business Management System
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Report / Technical Note

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Filtration and UV disinfection

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CONTENTS

TABLES

APPENDICES

APPENDIX A
Primary data on growth inhibition tests with the marine alga *Skeletonema costatum*

APPENDIX B
Primary data on larval development ratio tests with the crustacean *Acartia tonsa*

APPENDIX C
Primary data on short-term toxicity tests on embryo and sac-fry stages with the marine fish *Cyprinodon variegatus*

APPENDIX D
Certificate of compliance, ISO 9001 certificate, accreditation and GLP authorisation

Abbreviations

Abbreviation	Description
BWMS	Ballast water management system
DNV	Det Norske Veritas
EC	Effect Concentrations
IMO	International Maritime Organization
LOEC	Lowest Observed Effect Concentration
LC	Lethal Concentrations
MEPC	Marine Environment Protection Committee
NOEC	No Observed Effect Concentration
PSU	Practical salinity units
SOP	Standard operating procedure
STD	Standard deviation
T0	Day 0 samples
T5	Day 5 samples
TUc	Chronic Toxicity Unit
WET	Whole effluent toxicity

1 Executive summary and conclusion

In order to minimize the risk of spreading invasive species, the International Maritime Organization (IMO) has set out a mandatory framework for treatment of ballast water.

For ballast water management systems (BWMS) using an active substance, Whole Effluent Toxicity (WET) tests have to be conducted to assess the effects of treated ballast water on the receiving ecosystems (Resolution MEPC.169 (57) (G9) /1/). The basic treatment principles of RayClean are mechanical filtration and ultra violet (UV) radiation and, thus, the BWMS is not using an active substance that would imply an evaluation according to Resolution MEPC.169 (57) (G9) /1/. Nevertheless, DESMI Ocean Guard requested WET tests conducted according to G9 /1/.

DHI conducted biological efficacy performance evaluation of RayClean from March, 2013 to May, 2014 at the land-based test facility in Hundested, Denmark. Treated discharge water and control discharge water samples were collected from pilot test cycle B-1, conducted with brackish water during 21-26 March 2013, and test cycle B-6 which was conducted with brackish water during 15-20 May 2014 /6/. The use of the samples collected from the pilot test cycle for the WET tests was accepted by DNV GL.

The residual effects of RayClean-treated discharge water were analysed in WET tests with aquatic species representing three trophic levels. One primary producer (alga), one consumer (crustacean) and one predator (fish) were used for the tests. For each toxicity test, dilution series were prepared by mixing treated discharge water with control discharge water. Each test included two control series, one with control discharge water and one laboratory control prepared from natural seawater.

A summary of the obtained results is presented in Table 1.1.

Table 1.1 Chronic aquatic toxicity of treated discharge water

Test organism	Salinity	Standard/ guideline	NOEC (%)	LOEC (%)	L(E)C25 (%)	TUc (100/ L(E)C25)
Alga (*Skeletonema costatum*)	Brackish	ISO 10253 /3/	≥ 91	91	> 91	< 0.9
Crustacean (*Acartia tonsa*)	Brackish	ISO/FDIS 16778 /4/	< 25	25	> 100	< 1
Fish (*Cyprinodon variegatus*)	Brackish	OECD 212 /5/	≥ 100	100	> 100	< 1

The chronic test results are expressed by Toxic Units (TUc, Chronic Toxicity Unit), where TUc = 100/EC25. According to the EPA National Pollutant Discharge Elimination System for issuing of a Vessel General Permit /6/, a chronic toxicity of discharge or effluents from a vessel, which exceeds a value for TUc of 1.6, is considered toxic.The WET tests with algae and fish showed no toxic effects of the treated discharge water in the highest tested concentrations (91% and 100%, respectively). The WET test with crustaceans showed an effect on the larval development ratio as indicated by the NOEC and LOEC in Table 1.1. However, the TUc value, which was calculated for this test, was below 1.

The results of the WET tests confirmed that ballast water treated with RayClean under standard operating conditions as instructed by the manufacturer is not expected to cause chronic toxic effects to the aquatic environment.

2 Introduction

DHI conducted biological efficacy performance evaluation of RayClean from March, 2013 to May, 2014 at the land-based test facility in Hundested, Denmark.

For ballast water management systems (BWMS) using an active substance, WET tests have to be conducted to assess the effects of treated ballast water on the receiving ecosystems (Resolution MEPC.169 (57) (G9) /1/). The basic treatment principles of RayClean are mechanical filtration and ultra violet (UV) radiation and, thus, the BWMS is not using an active substance that would imply an evaluation according to Resolution MEPC.169 (57) (G9) /1/. Nevertheless, DESMI Ocean Guard requested WET tests conducted according to G9 /1/.

The purpose of the WET tests was to investigate possible toxic effects of BWMS treated discharge water according to the G9 requirements (Resolution MEPC.169 (57)) /1/. The potential residual toxic effects of RayClean-treated discharge water were analysed in WET tests with aquatic species representing three trophic levels. One marine primary producer (alga), one consumer (crustacean) and one predator (fish) were used for the tests.

All toxicity tests were performed by DHI Environmental Laboratory. DHI’s Environmental Laboratory holds an accreditation according to ISO 17025 which includes biological analyses related to performance evaluation of BWMS and ecotoxicological studies.

3 Materials and methods

3.1 Sampling of water and preparation of test concentrations

Treated discharge water and control discharge water samples were collected from pilot test cycle B-1, conducted with brackish water during 21-26 March 2013, and test cycle B-6 which was conducted with brackish water during 15-20 May 2014 /6/. The use of the samples collected from the pilot test cycle for the WET tests was accepted by DNV GL.

The samples were collected by personnel from DHI at the DHI test facility in Hundested, Denmark. Details of the sampling are summarized in Table 3.1.

Table 3.1 Parameters measured on discharge water for the pilot test cycle B-1 and the test cycle B-6 used for WET study

Test cycle	Inlet	Discharge
Pilot test B-1	2013.03.21	2013.03.26
B-6*	2014.05.15	2014.05.20

* *The validity criteria for the crustacean larval development ratio test with discharge water from pilot test cycle B-1 were not fulfilled and it was thus decided to repeat the test with control and treated discharge water from test cycle B-6.*

The treated discharge water and control discharge water samples were filtered using a GF/C filter and, subsequently, divided into subsamples for the WET tests. The samples were stored at DHI at -20°C until initiation of the tests. A DHI lab. No. was assigned to each sample (Table 3.2) and used to identify the samples during testing, data analysis and reporting.

Table 3.2 Samples collected for whole effluent toxicity tests

Treatment	Water type	Test cycle	Sampling date	Salinity (PSU*)	DHI lab. No.
RayClean BMWS	Brackish	Pilot test B-1	2013.03.26	19	13-0976
Control (no treatment)	Brackish	Pilot test B-1	2013.03.26	19	13-0977
RayClean BMWS	Brackish	B-6	2014.05.20	18	14-1016
Control (no treatment)	Brackish	B-6	2014.05.20	18	14-1015

* *Practical salinity unit*

For each toxicity test, dilution series were made by mixing treated discharge water with control discharge water. Control discharge water was used as control and dilution water because the purpose was to examine if the discharge water was toxic to aquatic organisms after treatment in the BWMS. The actual treatment is the only difference between the control and the treated discharge water. When possible, the WET tests were conducted with undiluted treated discharge water as the highest test concentration. However, for the algal test (ISO 10253 /1/), addition of nutrients is necessary and, thus, undiluted samples cannot be used for the testing of the toxicity. Therefore, the highest tested concentration in the algal test was 91%.

Each test included two control series, one with control discharge water and one laboratory control prepared from natural seawater without addition of any discharge water. The seawater used for the laboratory control was collected in the North Sea by the

Danish Institute for Fisheries Research and brought to DHI by tank lorry. The seawater (salinities of 29 practical salinity units (PSU) for the tests with algae and fish test and 32 PSU for the test with crustaceans) was filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm) before use. This seawater was diluted to attain a salinity of 18-20 PSU in the WET tests.

The concentrations used for the WET tests are given in Table 3.3.

Table 3.3 Chronic toxicity tests performed with treated discharge water samples

Trophic level	Species	Test	Standard/ guideline	Test duration	Test conc. (% treated discharge water)
Alga*	*Skeletonema costatum*	Growth inhibition test	ISO 10253 /1/	72 h	0; 25; 50 and 91
Crustacean**	*Acartia tonsa*	Larval development ratio test	ISO/FDIS 16778 /4/	5 d	0; 25; 50 and 100
Fish*	*Cyprinodon variegatus* (sheepshead minnow)	Fish, short-term toxicity test on embryo and sac-fry stages	OECD 212 /5/	12 d	0; 25; 50 and 100

* *Test performed with control and treated discharge water from pilot test cycle B-1*

** *Test performed with control and treated discharge water from test cycle B-6*

3.2 Aquatic toxicity tests

3.2.1 Study on the growth inhibition of the algae *Skeletonema costatum*

The toxicity of the treated discharge water and the control discharge water samples from pilot test cycle B-1 was determined as the growth inhibition of the marine alga *Skeletonema costatum* (clone: NIVA-BAC 1) according to the ISO International Standard 10253 "Water Quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*" /1/. The algae used in the test were cultured at a salinity of 20 PSU.

The test mixtures were prepared as serial dilutions of the treated discharge water with the control discharge water. No pH adjustment was necessary as the pH was within the required range. Nutrients, salts and algae were added to each of the test mixtures (i.e. each concentration of samples) according to ISO 10253 /1/. The solutions of nutrients and salts and added algae accounted for 90% of the test mixtures. The concentrations of the treated discharge water applied in the test were: 0 (laboratory control); 0 (control discharge water); 25; 50 and 91%.

The tests were performed in 250-mL glass flasks with wide neck, each containing 100 mL of test mixture. The test design included triplicate test flasks of each concentration of treated discharge water and algae, six test flasks containing control discharge water and algae, six laboratory controls containing seawater and algae and one blank control of each concentration (similar to dilution series of the treated discharge water, but without algae). The test mixtures were incubated for approx. 72 hours under continuous shaking and constant illumination from a panel of fluorescent light.

The test conditions are summarized in Table 3.4.

The density of the algae in the laboratory control was measured at test start by use of a Beckman Coulter Multisizer^{TM} 3 Coulter Counter$^{®}$. At the beginning of the test and after

24, 48 and 72 ± 2 hours of incubation, the algal growth was measured as in-vivo fluorescence in triplicate test flasks, the blanks and the six controls by use of a Turner TD-700 Laboratory Fluorometer. The identity and normal appearance of *Skeletonema costatum* in the laboratory control was confirmed by microscopy at the end of the test.

A test with the reference substance 3,5-dichlorophenol (3,5-DCP) was performed in order to verify the sensitivity of the algae.

Table 3.4 Test conditions for the growth inhibition test performed with *Skeletonema costatum*

Test guideline	ISO 10253 /1/
Test organism	*Skeletonema costatum*
Test organism source	Niva, Norway, cultured at DHI (clone: NIVA, BAC 1)
Test organism life stage	Log phase growth for approx. 3 days
Test duration	72 ± 2 hours
Test vessel	250-mL conical flask with air permeable lid
Initial target concentration	350 - 1,000 part/mL ~ 0.7 - 2.0 x 10^3 cell/mL
Replicates	6 × laboratory control 6 × control discharge water 3 × test concentration (treated discharge water) 1 x test group blank
Method	Determine reduction of specific growth rate by fluorescence measurements
End point	Growth rate
Laboratory control medium	Seawater (salinity 29) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm), adjusted to as salinity of 20 with MQ water and heated to 73 °C where after nutrients were added.
Nutrient media	According to ISO 10253 /1/
Photoperiod	Constant fluorescent light
Light intensity	Constant fluorescent light (60-120 µmol × m^{-2} × sec^{-1})
Shaking	120 rpm
Temperature	19 °C ± 1 °C
Initial pH	8.0 ± 0.2
Salinity	20 PSU
Validity criteria	72 hours specific growth ratio in controls ≥ 0.9 d^{-1} 72 hours pH increase in controls < 1.0 Control variation coefficient ≤ 7%
Reference test	Verification of organism sensitivity with 3,5-DCP

3.2.2 Calanoid copepod development test with *Acartia tonsa*

The treated discharge water samples from test cycle B-6 were tested for chronic toxic effects on *Acartia tonsa* according to the ISO/FDIS 16778 "Water quality - Calanoid copepod development test with *Acartia tonsa*" /4/.

The test mixtures were prepared as serial dilutions of the treated samples with the control discharge water. No pH adjustment was necessary as the pH was within the required range. The concentrations of the treated discharge water applied in the test were: 0 (laboratory control); 0 (control discharge water); 25, 50 and 100%.

A strain of *Acartia tonsa* (DANA), Copepoda, Crustacea, collected in the North Sea by the Danish Institute for Fisheries Research has been cultured at DHI since 1987. The test was initiated with eggs collected from this culture. The eggs used for the test were cultured at a salinity of 18 PSU.

The test was performed in 250-mL glass beakers. At the initiation of the test, 40 mL of test mixture and between 60 and 90 eggs (and the less possible newly hatched nauplii) were added to each beaker. *Rhodomonas salina* algae at 50,000 cells/mL were added as feed. On day 3, 40 mL of freshly prepared test mixture and *Rhodomonas salina* to a final concentration of approx. 50,000 cells/mL were added. On day 5, the test mixtures were filtered through 10-µm filters after fixation with a Lugol's solution. The filters were transferred to Petri dishes with marked graduations and the numbers of nauplii, copepodites and non-hatched eggs were counted.

The larval development test was performed with 10 replicates for each test concentration and 12 control replicates (control discharge water) and 6 laboratory control replicates.

The test conditions are summarized in Table 3.5.

The pH, salinity and dissolved oxygen were measured at the test start, before and after renewal on day 3 and at the end of the test.

The effect parameter determined and used for evaluation of possible toxic effects is the larval development ratio. Furthermore, hatching rate and mortality rate were determined after exposure.

The larval development ratio was calculated from the number of surviving larvae (the ratio of copepodites to the sum of nauplii and copepodites) and, thus, the LDR expresses the progress of larval development:

$$LDR = \left(\sum_{R} \frac{Copepodites_{te,r}}{Nauplii_{te,r} + Copepodites_{te,r}}\right) \times \frac{1}{R}$$

Where:

- ***R***: number of replicates used for each test group.
- ***Nauplii*** $_{t\,e,\,r}$: number of nauplii in each replicate at the end point (t_e), in each replicate (r).
- ***Copepodites*** $_{t\,e,\,r}$: number of copepodites at the end point (t_e), in each replicate (r).

Table 3.5 Test conditions for the larval development test with *Acartia tonsa*

Test guideline	ISO/FDIS 16778 "Water quality - Calanoid copepod early-life stage test with *Acartia tonsa* /4/	
Test organism source	DHI (collected in the North Sea by the Danish Institute for Fisheries Research, cultured at DHI since 1987)	
Test organism life stage	Eggs collected from the *A. tonsa* culture maintained at DHI laboratory	
Test duration	Up to 6 days	
Test vessel	250-mL beaker	
Replicates	12 × control discharge water 10 × test concentration (treated discharge water) 6 × laboratory control	
Test organisms/ test vessel	60 – 90	
End points	Early Life Stage Mortality in the control (ELS) Hatching Success (HS) Larval Development Ratio (LDR)	
Laboratory control medium	Seawater (salinity 32) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm) and adjusted to a salinity of 18	
Food regime	*Rhodomonas salina* 50,000 cells/mL	
Photoperiod	16:8 h	
Temperature	20 °C ± 1 °C	
pH during testing	8.00 ± 0.3 pH units	
Salinity (PSU)	18	
Validity criteria	Early Life Stage Mortality in the control (ELS)	< 30%
	Hatching Success in the control (HS)	> 75%
	Larval Development Ratio in the control (LDR)	≥ 60% ± 20%
	Dissolved oxygen concentration (DO)	> 70% throughout test
	Temperature (T)	20 °C ± 1 °C
	Salinity variation from the control start (S_i) value	± 2‰

3.2.3 Fish, short-term toxicity test on embryo and sac-fry stages with *Cyprinodon variegatus*

The treated discharge water sample from pilot test cycle B-1 was tested for the chronic toxic effects on embryo and sac-fry stages of *Cyprinodon variegatus* (sheepshead minnow) by exposure of the life stages from the fertilized egg to the end of the sac-fry stage in accordance with the OECD Guideline for the Testing of Chemicals No. 212 /5/. The eggs were deposited at a salinity of 18 PSU.

The test mixtures were prepared as serial dilutions of the treated discharge water with the control discharge water. No pH adjustment was necessary as the pH was within the required range. The concentrations of treated discharge water applied in the test were: 0 (laboratory control); 0 (control discharge water); 25; 50 and 100%.

The test was started with fertilized eggs in the test chambers and was terminated just before the yolk-sac of any larvae in any of the test chambers was completely absorbed or before mortality by starvation started in laboratory controls. The test was carried out as a semi-static test in 175-mL glass beakers with 150 mL of test mixture. Thirty eggs, equally distributed between three replicates, were exposed at each concentration of treated discharge water, control discharge water and in the parallel laboratory control.

The test conditions are summarized in Table 3.6.

Test mixtures were renewed on day 4 and subsequently three times a week by preparing fresh test mixtures in clean vessels, after which surviving eggs and larvae were gently transferred to the new vessels in a small volume of old solution, avoiding exposure to air.

The salinity, pH, water temperature and dissolved oxygen were measured at the test start, before and after each water renewal and at the end of the test. The room temperature was measured continuously by a thermologger.

No feed was provided in the embryo and sac-fry test as the test was terminated while the fry were still nourished from the yolk sac.

Table 3.6 Summary of test conditions for the chronic toxicity test with *Cyprinodon variegatus*

Test guideline	OECD 212 "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages"/5/	
Test organism	*Cyprinodon variegatus* (Sheepshead minnow)	
Test organism source	Aquatic Research Organisms, Inc. (ARO), USA	
Test organism life stage	Eggs (deposited 3 days before test start at approx.. 20 PSU)	
Test duration	12 days	
Test vessel	175-mL glass beakers (150 mL test solution)	
Replicates	3	
Test eggs/test vessel	10	
Test eggs/concentration	30	
End points	Hatching, mortality, malformation and visible abnormalities, length	
Laboratory control medium	Seawater (salinity 29) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm) and adjusted to a salinity of 19 with zebra fish test medium	
Food regime	No food during testing	
Photoperiod	12:12 h	
Temperature	24.0 °C ± 1.5 °C	
pH during testing	8.0 ± 0.5 pH units	
Salinity (PSU)	18	
Validity criteria	Survival of hatched larvae in the control (S)	> 80%
	Hatching Success in the control (HS)	> 75%
	Dissolved oxygen concentration (DO)	> 60
	Water temperature differing between test chambers or between successive days (T)	24 °C ± 1.5 °C

4 Calculations

In the alga test the LOEC/NOEC values were determined by use of Dunnett's procedure /7/. The EC values were calculated by use of the computer program TOXEDO /8/. The NOEC values in the fish and crustacean test was determined by use of a student's t-test ($p<0.05$) as the highest tested concentration, at which no significant effect was observed compared with the control and the LOEC is the concentration just above the NOEC. The pragmatic approach of calculation of residual toxicity of discharged ballast water suggested by US EPA /6/ was applied. The chronic test results are expressed by Toxic Units (TUc, Chronic Toxicity Unit), where TUc = 100/EC25.

5 Results

5.1 Overview

The results obtained in the WET tests were used to derive the no observed effect concentration (NOEC), lowest observed effect concentration (LOEC) and the effect concentrations (EC) or lethal concentrations (LC) causing 25% effect in comparison with the control (i.e. EC25 or LC25). A TUc value was then estimated by use of the EC25 or LC25 (see Section 4). A summary of the results of the toxicity tests with treated discharge water is presented in Table 5.1.

Table 5.1 Chronic aquatic toxicity of treated discharge water

Test organism	Standard/ guideline	NOEC (%)	LOEC (%)	L(E)C25 (%)	TUc (100/ L(E)C25)
Alga (*Skeletonema costatum*)	ISO 10253 /1/	≥ 91	91	> 91	< 1.1
Crustacean (*Acartia tonsa*)	ISO/FDIS 16778 /4/	< 25	25	> 100	< 1
Fish (*Cyprinodon variegatus*)	OECD 212 /5/	≥ 100	100	> 100	< 1

5.2 Algal growth inhibition test with *Skeletonema costatum*

The primary data from the growth inhibition test with the alga *Skeletonema costatum* are presented in Appendix A and the results are summarized in Table 5.2.

No toxicity was observed for the treated discharge water as the NOEC was ≥ 91 % (the highest tested concentration).

As presented in Table 5.2, all validity criteria were fulfilled. The reference test with 3,5-DCP resulted in an EC50 of 1.70 (1.63-1.79) mg/L, which is within the expected range set in the ISO standard /1/ (1.6 (1.0-2.2) mg/L). This indicates good test procedures and normal sensitivity of test organisms (Appendix A.2.2).

Table 5.2 Results of the *Skeletonema costatum* growth inhibition test with treated discharge water

Test water	NOEC (%)	LOEC (%)	EC25 (%)	TUc (100/ EC25)	Validity criteria		
					Control pH increase during test	Control specific growth rate	Control variation coefficient
Treated discharge water	≥ 91	91	> 91	< 1.1	< 1.0 (observed: 0.7)	> 0.9 per day (observed: 2.06 per day)	≤ 7% (observed: 2.8%)

5.3 Calanoid copepod development test with the crustacean *Acartia tonsa*

The primary data from the chronic toxicity test with the crustacean *Acartia tonsa* are presented in Appendix B and the results are summarized in Table 5.6.

Significant differences were observed on the larval development ratio (LDR) for all dilutions of the treated discharge compared with the control discharge water group (Appendix B.2 and Table 5.3).

The LDR was lower in the undiluted treated discharge water (concentration: 100%), but also at the highest dilution (concentration: 25%). The observed LDR of the treated discharge water (68.4%) was lower than the LDR observed with the control discharge water (75.5%). The LOEC for larval development was, therefore, equivalent to the lowest tested concentration of 25%, whereas the NOEC was estimated to be below 25%.

The data did not allow statistical calculation to establish a dose-response curve within 95% confidence limits, and the EC2525 value could not be calculated (Appendix B.7.1).

Table 5.3 Effects on the larval development ratio after exposure to treated discharge water

Test water	Concentration (%)	Larval development ratio mean	Standard deviation
Control discharge water	0	75.5	6.0
Treated discharge water	25	68.4*	9.0
Treated discharge water	50	66.5*	5.4
Treated discharge water	100	61.3*	6.8
Laboratory control	0	66.5*	5.7

* The mean for this conc. is significantly less than the control mean at alpha = 0.05 (1-sided) by a t-test with Bonferroni adjustment of alpha level.

Significant differences were observed on the hatching success for the 50% and 100% concentrations of the treated discharge water when compared with the control discharge water group (Appendix B.3 and Table 5.4).

The LOEC for hatching was the 50% concentration, whereas the NOEC was estimated to be the 25% concentration of treated discharge water.

The data did not allow statistical calculation to establish a dose-response curve within 95% confidence limits, and the EC2525 value could not be calculated (Appendix B.7.2).

Table 5.4 Effects on the hatching success after exposure to treated discharge water

Test water	Concentration (%)	Hatching success mean	Standard deviation
Control discharge water	0	93.5	2.9
Treated discharge water	25	91.0	4.0
Treated discharge water	50	89.2*	4.5
Treated discharge water	100	90.8*	3.2
Laboratory control	0	93.5	0.6

* The mean for this conc. is significantly less than the control mean at alpha = 0.05 (1-sided) by a t-test with Bonferroni adjustment of alpha level. Hatching success is 2.7% lower in the undiluted treated discharge group than in the control discharge group.

No significant difference was observed on the mortality of the early life stages in the undiluted treated discharge water when compared with the control discharge water group (Appendix B.7.3 and Table 5.5). Therefore, the LOEC and EC2525 for effects on early life stages could not be calculated. The NOEC for effects on early life stages was estimated to be above the highest tested concentration.

Table 5.5 Effects on the ELS mortality after exposure to treated discharge water

Test water	Concentration (%)	ELS Mortality	Standard deviation
Control discharge water	0	19.4	8.8
Treated discharge water	25	21.9	4.6
Treated discharge water	50	13.4	6.1
Treated discharge water	100	16.9	2.6
Laboratory control	0	26.4	13.4

Table 5.6 NOEC, LOEC, EC2525 and TUc values obtained in the *Acartia tonsa* chronic toxicity test with treated discharge water for the different endpoints

End points	Test water	NOEC (%)	LOEC (%)	EC25 (%)	TUc (100/EC25)
Larval development ratio	Treated discharge	< 25	25	> 100	≤ 1
Hatching success	Treated discharge	25	50	> 100	≤ 1
Early life stage mortality	Treated discharge	≥ 100	100	> 100	≤ 1

These differences observed between the treated discharge water and the control discharge water groups of *A. tonsa* are unlikely to be due to the exposure to the treated discharge water, as there was no significant dose-response relations between the examined concentrations tested for both the LDR (Table 5.3) and the hatching success (Table 5.4) endpoints.

Furthermore, the LDR in the laboratory control group, which was exposed to natural seawater, was in the same range as the LDR in the treated water discharge groups.

The average inhibition of the hatching success after exposure to the undiluted treated discharge water (concentration: 100%) was 2.7% (see note below Table 5.4). This low inhibition without a significant dose-response is not considered as an effect of the treated discharge water.

All validity criteria given in Table 3.5 for the test were fulfilled (see the table in Appendix B.6).

5.4 Fish, short-term toxicity test on embryo and sac-fry stages with *Cyprinodon variegatus*

The primary data from the chronic toxicity test with the fish *Cyprinodon variegatus* are presented in Appendix C and the results are summarized in Table 5.7.

Significant differences were observed in the size of the fish larvae (growth end point) after exposure to the 25% and 50% concentrations the treated discharge water when compared with the control discharge water group (Appendix C.4.4). However, no difference was observed in the size of the larvae after exposure to the undiluted treated discharge water (concentration: 100%) when compared with the control discharge water group. The LOEC and NOEC were, therefore, estimated to be above the highest tested concentration of 100% treated discharge water.

The data did not allow statistical calculation to establish a dose-response curve within 95% confidence limits, and the EC2525 value could not be calculated.

No significant differences between exposures to treated discharge water and control discharge water were observed for any of the evaluated endpoints (embryonic mortality, larval mortality and overall mortality), and, hence, the NOECs were determined to be ≥ 100%.

The validity criteria given in Table 3.6 were fulfilled (see Appendix C.3).

Table 5.7 NOEC and LOEC values and EC25 and TUc values obtained in the *Cyprinodon variegatus* chronic toxicity test with treated discharge water for the different endpoints

End points	Test water	NOEC (%)	LOEC (%)	EC25 (%)	TUc (100/EC25)
Embryonic mortality	Treated discharge	≥ 100	100	> 100	≤ 1
Larval mortality	Treated discharge	≥ 100	100	> 100	≤ 1
Overall mortality	Treated discharge	≥ 100	100	> 100	≤ 1
Growth	Treated discharge	≥ 100	100	> 100	≤ 1

6 References

/1/ IMO (2008). Procedure for approval of ballast water management systems that make use of active substances (G9). Resolution MEPC.169 (57).

/2/ Performance evaluation in land based test facility of RayClean Ballast Water management system – DESMI Ocean Guard A/S, 2014.

/3/ DS/EN ISO 10253 (2006):"Water Quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*". Second edition. 2006.04.15.

/4/ ISO/CD 16778:"Water quality - Calanoid copepod early-life stage test with *Acartia tonsa*", March 2012.

/5/ OECD Guideline for Testing of Chemicals No. 212 (1998): "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages". Adopted 1998.09.21.

/6/ U.S. Environmental Protection Agency, Vessel General Permit for Discharges Incidental to the Normal Operation of Vessels (VGP). Vessel General Permit (VGP) Version 2/5/2009, Section 5.8; and Appendix J available at http://www.epa.gov/npdes/pubs/vessel_vgp_permit.pdf.

/7/ US-EPA (1994): Dunnett's Program Version 1.5. US Environmental Protection Agency, Cincinnati.

/8/ VKI (1999): TOXEDO Ver. 1.5. Program for statistical estimation of EC values, based on experimental data from ecotoxicological assays

APPENDIX A

Primary data on growth inhibition tests with the marine alga *Skeletonema costatum*

A Primary data on growth inhibition tests with the marine alga *Skeletonema costatum*

A.1 Primary data on fluorescence

Raw data generated in the algal growth inhibition test with Treated discharge

Replicate	Test concentration	Fluorescence measurements corrected for blank values			
	%	Start	26.5 h	49 h	72 h
Control discharge A	0	2.7	20.6	171	1333
Control discharge B	0	2.7	18.6	135	1009
Control discharge C	0	2.7	20.8	193	1306
Control discharge D	0	2.7	22	211	1459
Control discharge E	0	2.7	18.9	156	1270
Control discharge F	0	2.7	17.8	132	1018
Average	0	2.7	19.8	166	1232
A	25	2.7	14.7	132	895
B	25	2.7	19.6	171	1233
C	25	2.7	22.2	208	1458
Average	25	2.7	18.8	171	1195
A	50	2.7	15.9	175	1117
B	50	2.7	11.3	131	917
C	50	2.7	15.7	146	1072
Average	50	2.7	14.3	151	1035
A	91	2.7	18.8	185	1178
B	91	2.7	20.3	193	1385
C	91	2.7	16.3	137	1007
Average	91	2.7	18.5	172	1190

Measurements of pH and salinity

Date	2013.07.23		2013.07.26
Concentration (%)	Day 0		Day 3
	pH	Sal. (PSU)	pH
Control discharge water	8.2	18.3	9.0
Treated discharge water 25%	8.2	18.5	8.7
Treated discharge water 50%	8.2	18.8	8.8
Treated discharge water 91%	8.2	18.8	8.9
Laboratory control	8.1	20.4	8.8

A.2 Statistics

A.2.1 Inhibition of the growth of Skeletonema costatum with treated discharge water

Statistical parameters calculated from continuous responses based on continuous mean
Test type: Growth inhibition test.

Control values

Control discharge	Growth per hour	Inhibition in per cent
Control 1	0.087	-
Control 2	0.083	-
Control 3	0.087	-
Control 4	0.089	-
Control 5	0.086	-
Control 6	0.083	-
Control mean	0.086	0

Experimental data

Treated discharge concentration in %	Growth per hour	Inhibition in per cent
25	0.082	4
25	0.086	0
25	0.088	0
50	0.086	0
50	0.083	3
50	0.084	2
91	0.086	0
91	0.088	0
910	0.083	3

Dunnett’s procedure:

NOEC: ≥ 91%
LOEC: 91%

Results

Data did not allow statistical calculation of the EC values

EC10: > 91%
EC25: > 91%
EC50: > 91%

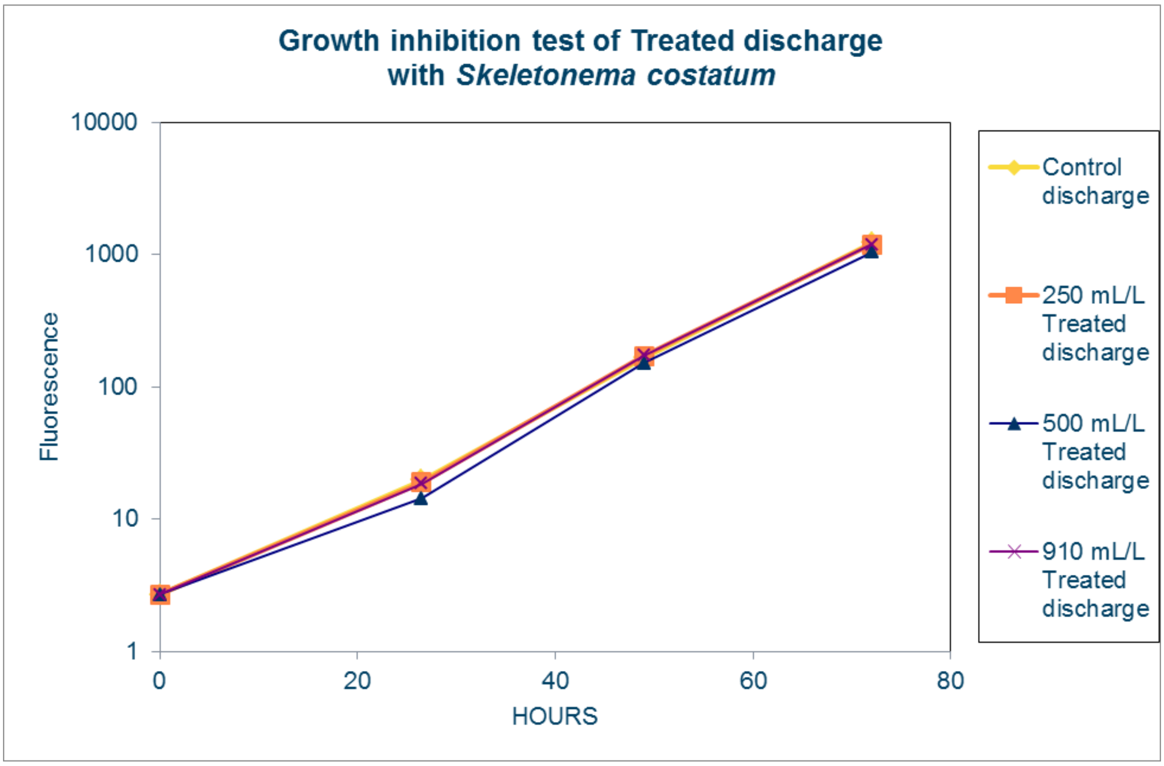


Figure A.1 Fluorescence against time (0-72 hours) with "Treated discharge water" with *Skeletonema costatum*

A.2.2 Inhibition of the growth of Skeletonema costatum with 3.5-dichlorophenol

Statistical parameters calculated from continuous responses based on continuous mean
Test type: Growth inhibition test

Control values

Concentration in mg/L	Growth per hour	Inhibition in per cent
Control 1	0.091	-
Control 2	0.090	-
Control 3	0.089	-
Control 4	0.087	-
Control 5	0.092	-
Control 6	0.089	-
Control mean	0.090	0

Experimental data

Concentration in mg/L	Growth per hour	Inhibition in per cent
0.4	0.089	1
0.4	0.091	0
0.7	0.091	0
0.7	0.085	5
1.0	0.079	12
1.0	0.083	7
1.4	0.059	34
1.4	0.065	28
2.0	0.030	67
2.0	0.032	64
2.8	0.001	99
2.8	0.001	99

Dunnett's procedure:

NOEC: 0.7 mg/L
LOEC: 1.0 mg/L

EC values and limits of the 95% confidence interval (LCL and UCL)

y(EC)	LCL	EC(yo)	UCL
10	0.89	0.99	1.07
50	1.63	1.70	1.79
90	2.66	>2.80	

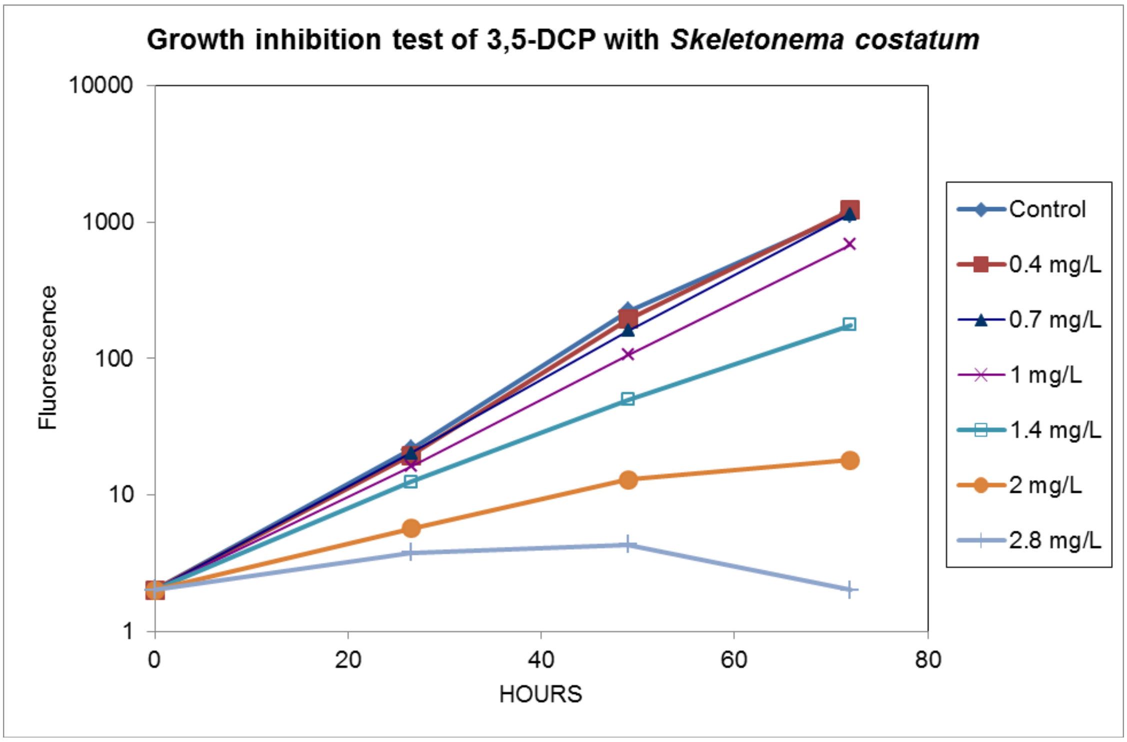
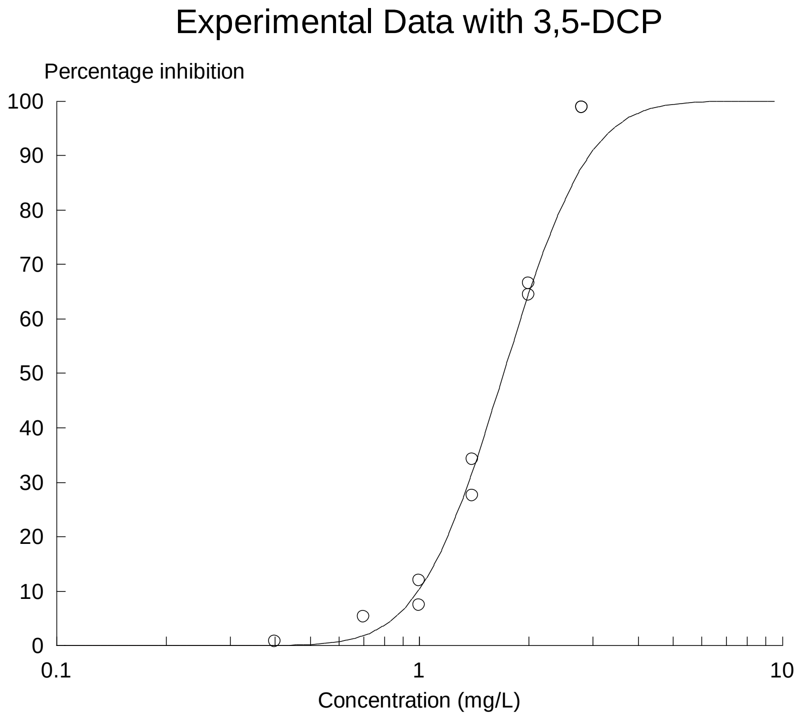


Figure A.2 Fluorescence against time (0-72 hours) 3.5-dichlorophenol with *Skeletonema costatum*



APPENDIX B

Primary data on larval development ratio tests with the crustacean *Acartia tonsa*

B Primary data on larval development ratio tests with the crustacean Acartia tonsa

B.1 Larval development and growth test (F0 generation)

Concentration		Start			End of exposure						
		Eggs added (+ nauplii added)		eggs + nauplii added	Eggs	Nauplii N	Cope-podites C	LDR C/(N+C)	Hatching success	ELS mortality	ELS survival
		Eggs	N	Number	Number			%			
Control discharge water	A	74	6	80	3	15	44	74.6	95.9	23.4	76.6
	B	80	1	81	1	20	44	68.8	98.8	20.0	80.0
	C	87	2	89	4	12	55	82.1	95.4	21.2	78.8
	D	61	2	63	6	12	37	75.5	90.2	14.0	86.0
	E	64	1	65	6	12	44	78.6	90.6	5.1	94.9
	F										
	G	88	1	89	4	18	40	69.0	95.5	31.8	68.2
	H	76	3	79	5	20	37	64.9	93.4	23.0	77.0
	I	65	2	67	3	11	31	73.8	95.4	34.4	65.6
	J	74	2	76	5	10	51	83.6	93.2	14.1	85.9
	K	69	3	72	7	11	48	81.4	89.9	9.2	90.8
	L	86	1	87	8	14	51	78.5	90.7	17.7	82.3
Treated discharge water 25%	A	60	2	62	5	17	30	63.8	91.7	17.5	82.5
	B	84	4	88	9	15	49	76.6	89.3	19.0	81.0
	C	66	0	66	7	13	33	71.7	89.4	22.0	78.0
	D	64	2	66	8	16	30	65.2	87.5	20.7	79.3
	E	69	2	71	0	15	36	70.6	100	28.2	71.8
	F	67	1	68	6	17	26	60.5	91.0	30.6	69.4
	G										
	H	69	1	70	6	12	38	76.0	91.3	21.9	78.1
	I	82	2	84	6	30	32	51.6	92.7	20.5	79.5
	J	72	3	75	10	11	43	79.6	86.1	16.9	83.1
Treated discharge water 50%	A	74	1	75	7	23	41	64.1	90.5	5.9	94.1
	B	81	0	81	17	23	31	57.4	79.0	15.6	84.4
	C	74	0	74	10	16	41	71.9	86.5	10.9	89.1
	D	71	2	73	10	14	43	75.4	85.9	9.5	90.5
	E	74	1	75	4	18	41	69.5	94.6	16.9	83.1
	F	85	2	87	9	25	44	63.8	89.4	11.5	88.5
	G	88	2	90	7	22	55	71.4	92.0	7.2	92.8
	H	65	2	67	4	17	32	65.3	93.8	22.2	77.8
	I	65	1	66	7	17	28	62.2	89.2	23.7	76.3
	J	74	1	75	7	22	39	63.9	90.5	10.3	89.7
Treated discharge water 100%	A	71	4	75	6	19	40	67.8	91.5	14.5	85.5
	B	84	0	84	6	30	33	52.4	92.9	19.2	80.8
	C	75	0	75	10	22	31	58.5	86.7	18.5	81.5
	D	71	1	72	6	13	41	75.9	91.6	18.2	81.8
	E	77	2	79	3	25	38	60.3	96.1	17.1	82.9
	F	76	0	76	6	24	35	59.3	92.1	15.7	84.3
	G	89	0	89	7	28	41	59.4	92.1	15.9	84.1
	H										
	I	75	1	76	10	25	33	56.9	86.7	12.1	87.9

Concentration		Start			End of exposure						
		Eggs added (+ nauplii added)		eggs + nauplii added	Eggs	Nauplii N	Cope-podites C	LDR C/ (N+C)	Hatching success	ELS mortality	ELS survival
		Eggs	N	Number	Number			%			
	J	64	2	66	8	18	28	60.9	87.5	20.7	79.3
Laboratory control	A	82	2	84	6	14	36	72.0	92.7	35.9	64.1
	B	80	4	84	5	27	40	59.7	93.8	15.2	84.8
	C	77	4	81	5	15	41	73.2	93.5	26.3	73.7
	D	71	3	74	5	23	42	64.6	93.0	5.8	94.2
	E	87	2	89	5	17	37	68.5	94.3	35.7	64.3
	F	79	2	81	5	18	28	60.9	93.7	39.5	60.5
Hatching control	A	79	1	80	10	57	1	-	87.3	-	-
	B	77	3	80	4	69	0	-	94.8	-	-
	C	78	2	80	10	64	0	-	87.2	-	-
	D	84	0	84	8	64	0	-	90.5	-	-

B.2 Larval development ratio

Replicate No.	Control discharge water	Treated discharge water 25%	Treated discharge water 50%	Treated discharge water 100%	Laboratory control
	Larval development ratio (%)				
1	74.6	63.8	64.1	67.8	72.0
2	68.8	76.6	57.4	52.4	59.7
3	82.1	71.7	71.9	58.5	73.2
4	75.5	65.2	75.4	75.9	64.6
5	78.6	70.6	69.5	60.3	68.5
6		60.5	63.8	59.3	60.9
7	69.0		71.4	59.4	
8	64.9	76.0	65.3		
9	73.8	51.6	62.2	56.9	
10	83.6	79.6	63.9	60.9	
11	81.4				
12	78.5				
Mean	75.5	68.4	66.5	61.3	66.5
Standard deviation	6.0	9.0	5.4	6.8	5.7
P-value	-	0.02	0.00	0.00	0.00
Significantly lower larval development ratio	-	YES	YES	YES	YES

B.3 Hatching success

Replicate No.	Control discharge water	Treated discharge water 25%	Treated discharge water 50%	Treated discharge water 100%	Laboratory control
	Hatching success (%)				
1	95.9	91.7	90.5	91.5	92.7
2	98.8	89.3	79.0	92.9	93.8
3	95.4	89.4	86.5	86.7	93.5
4	90.2	87.5	85.9	91.5	92.9
5	90.6	100.0	94.6	96.1	94.3
6	95.5	91.0	89.4	92.1	93.7
7	93.4	91.3	92.0	92.1	
8	95.4	92.7	93.8	86.7	
9	93.2	86.1	89.2	87.5	
10	89.9		90.5		
11	90.7				
12					
Mean	93.5	91.0	89.2	90.8	93.5
Standard deviation	2.9	4.0	4.5	3.2	0.6
P-value	-	0.06	0.01	0.03	0.48
Significantly lower hatching success rate	-	NO	YES	YES	NO

B.4 Early Life Stage mortality

Replicate No.	Control discharge water	Treated discharge water 25%	Treated discharge water 50%	Treated discharge water 100%	Laboratory control
	ELS Mortality (%)				
1	23.4	17.5	5.9	14.5	35.9
2	20.0	19.0	15.6	19.2	15.2
3	21.2	22.0	10.9	18.5	26.3
4	14.0	20.7	9.5	18.2	5.8
5	5.1	28.2	16.9	17.1	35.7
6	31.8	30.7	11.5	15.7	39.5
7	23.0	21.9	7.2	15.9	
8	34.4	20.5	22.2	12.1	
9	14.1	16.9	23.7	20.7	
10	9.2		10.3		
11	17.7				
12					
Mean	19.4	21.9	13.4	16.9	26.4
Standard deviation	8.8	4.6	6.1	2.6	13.4
P-value	-	0.23	0.04	0.21	0.11
Significantly higher mortality rate	-	NO	NO	NO	NO

B.5 Measurements of pH, oxygen and salinity

Date	2014.06.13			2014.06.16			2014.06.16			2014.06.18		
Concentration (%)	Day 0			Day 3 before addition			Day 3 after addition			Day 5		
	pH	O_2 (%)	Sal. (PSU)	pH	O_2 (%)	Sal. (PSU)	pH	O_2 (%)	Sal. (PSU)	pH	O_2 (%)	Sal. (PSU)
Control discharge water	8.3	96	17.9	8.5	99	18.1	8.3	95	18.0	8.4	100	18.2
Treated discharge water 25%	8.2	98	18.0	8.3	97	18.2	8.2	94	18.1	8.4	100	18.2
Treated discharge water 50%	8.2	98	18.0	8.3	97	18.2	8.2	94	18.1	8.4	100	18.2
Treated discharge water 100%	8.2	98	18.0	8.4	97	18.2	8.3	95	18.1	8.4	100	18.2
Laboratory control	7.9	100	17.9	8.7	100	18.0	8.3	100	17.9	8.6	100	18.1

B.6 Validity criteria

Date	Criteria	Target Value	Fulfilled
Validity criteria	Early Life Stage Mortality in the control (ELS)	ELS < 30%	YES
	Hatching Success in the control (HS)	HS > 75%	YES
	Larval Development Ratio in the control (LDR)	LDR = 60% ± 20%	YES
	Dissolved oxygen concentration (DO)	DO > 70% throughout test	YES
	Temperature (T)	T = 20 °C ± 1 °C	YES
	Salinity variation from the control start (S_i) value	S_i‰ ± 2‰	YES

B.7 Statistics

B.7.1 Inhibition of the larval development ratio (LDR) of Acartia tonsa with Treated discharge (14-1016)

Statistical parameters calculated from continuous responses based on continuous mean
Test type: *Acartia tonsa* early-life stage test

Control values

Control discharge (14-1015)	Larval Development Ratio	Inhibition
-	(%)	(%)
Control 1	74.6	-
Control 2	68.8	-
Control 3	82.1	-
Control 4	75.5	-
Control 5	78.6	-
Control 6	69.0	-
Control 7	64.9	-
Control 8	73.8	-
Control 9	83.6	-
Control 10	81.4	-
Control 11	78.5	-
Control mean	75.5	-

Experimental data

Treated discharge (14-1016)	Larval Development Ratio	Inhibition
(%)	(%)	(%)
25	63.8	16
25	76.6	0
25	71.7	5
25	65.2	14
25	70.6	7
25	60.5	20
25	76.0	0
25	51.6	32
25	79.6	0
50	64.1	15
50	57.4	24
50	71.9	5
50	75.4	0

50	69.5	8
50	63.8	16
50	71.4	5
50	65.3	14
50	62.2	18
50	63.9	15
100	67.8	10
100	52.4	31
100	58.5	23
100	75.9	0
100	60.3	20
100	59.3	21
100	59.4	21
100	56.9	25
100	60.9	19

Results

Data did not allow statistical calculation of the 95% confidence limits

EC10: N/A

EC25: > 100%

EC50: > 100%

t-test:

Significantly lower larval development ratio was found at 25% treated discharge compared to control discharge.

B.7.2 Inhibition of the hatching success of Acartia tonsa with Treated discharge (14-1016)

Statistical parameters calculated from continuous responses based on continuous mean
Test type: *Acartia tonsa* early-life stage test

Control values

Control discharge (14-1015)	Hatching success	Inhibition
-	(%)	(%)
Control 1	95.9	-
Control 2	98.8	-
Control 3	95.4	-
Control 4	90.2	-
Control 5	90.6	-
Control 6	95.5	-
Control 7	93.4	-
Control 8	95.4	-
Control 9	93.2	-
Control 10	89.9	-
Control 11	90.7	-
Control mean	93.5	-

Experimental data

Treated discharge (14-1016)	Hatching success	Inhibition
(%)	(%)	(%)
25	91.7	2
25	89.3	5
25	89.4	4
25	87.5	6
25	100.0	0
25	91.0	3
25	91.3	2
25	92.7	1
25	86.1	8
50	90.5	3
50	79.0	16
50	86.5	8
50	85.9	8
50	94.6	0
50	89.4	4
50	92.0	2
50	93.8	0
50	89.2	5
50	90.5	3
100	91.5	2
100	92.9	1
100	86.7	7
100	91.5	2
100	96.1	0
100	92.1	2
100	92.1	2
100	86.7	7
100	87.5	6

Results

Data did not allow statistical calculation of the EC values

EC10: > 100%

EC25: > 100%

EC50: > 100%

t-test:

Significantly lower hatching success was found at 50% treated discharge compared to control discharge.

B.7.3 Inhibition of the survival of the early-life stage (ELS) of Acartia tonsa with Treated discharge (14-1016)

Statistical parameters calculated from continuous responses based on continuous mean
Test type: *Acartia tonsa* early-life stage test

Control values

Control discharge (14-1015)	Survival of the ELS	Inhibition
-	(%)	(%)
Control 1	76.6	-
Control 2	80.0	-
Control 3	78.8	-
Control 4	86.0	-
Control 5	94.9	-
Control 6	68.2	-
Control 7	77.0	-
Control 8	65.6	-
Control 9	85.9	-
Control 10	90.8	-
Control 11	82.3	-
Control mean	80.6	-

Experimental data

Treated discharge (14-1016)	Survival of the ELS	Inhibition
(%)	(%)	(%)
25	82.5	0
25	81.0	0
25	78.0	3
25	79.3	2
25	71.8	11
25	69.4	14
25	78.1	3
25	79.5	1
25	83.1	0
50	94.1	0
50	84.4	0
50	89.1	0
50	90.5	0
50	83.1	0
50	88.5	0
50	92.8	0
50	77.8	3
50	76.3	5
50	89.7	0
100	85.5	0
100	80.8	0
100	81.5	0
100	81.8	0
100	82.9	0
100	84.3	0
100	84.1	0
100	87.9	0
100	79.3	2

Results

Data did not allow statistical calculation of the EC values

EC10: > 100%

EC25: > 100%

EC50: > 100%

t-test:

A significantly different survival of the early-life stage was not seen at 100% treated discharge compared to control discharge.

APPENDIX C

Primary data on short-term toxicity tests on embryo and sac-fry stages with the marine fish *Cyprinodon variegatus*

C Primary data on short-term toxicity tests on embryo and sac-fry stages with the marine fish *Cyprinodon variegatus*

C.1 Primary data on embryo and on larvae observations with *Cyprinodon variegatus*

DATE		2013.07.25 - Day 0											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	-	-	-	-	-	-	0	0	0	0	0	0
Control discharge water	30	-	-	-	-	-	-	0	0	0	0	0	0
Treated discharge water 25%	30	-	-	-	-	-	-	0	0	0	0	0	0
Treated discharge water 50%	30	-	-	-	-	-	-	0	0	0	0	0	0
Treated discharge water 100%	30	-	-	-	-	-	-	0	0	0	0	0	0

DATE		2013.07.26 - Day 1											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	-	-	-	-	-	-	0	0	0	0	0	0
Control discharge water	30	-	-	-	-	-	-	0	0	0	0	0	0
Treated discharge water 25%	30	-	-	-	-	-	-	0	0	0	0	0	0
Treated discharge water 50%	30	-	-	-	-	-	-	0	0	0	0	0	0
Treated discharge water 100%	30	-	-	-	-	-	-	0	0	0	0	0	0

DATE		2013.07.28 - Day 3											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	0	0	0	0	0	0
Control discharge water	30	0	0	0	0	0	0	0	0	0	0	0	0
Treated discharge water 25%	30	0	0	0	0	0	0	1	0	0	1	0	0
Treated discharge water 50%	30	0	0	0	0	0	0	1	1	0	1	1	0
Treated discharge water 100%	30	0	0	0	0	0	0	1	0	0	1	0	0

DATE		2013.07.29 - Day 4											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	5	5	2	5	5	2
Control discharge water	30	0	0	0	0	0	0	6	6	5	6	6	5
Treated discharge water 25%	30	0	0	0	0	0	0	6	4	6	6	4	6
Treated discharge water 50%	30	0	0	0	0	0	0	4	8	6	4	8	6
Treated discharge water 100%	30	0	0	0	0	0	0	8	5	6	8	5	6

DATE		2013.07.30 - Day 5											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	8	10	9	8	10	9
Control discharge water	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 25%	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	9	10	10	9	10

DATE		2013.07.31 - Day 6											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	10	10	10
Control discharge water	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 25%	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	9	10	10	9	10

DATE		2013.08.01 - Day 7											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	10	10	10
Control discharge water	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 25%	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	10	10	10	10	10

DATE		2013.08.02 - Day 8											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	10	10	10
Control discharge water	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 25%	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	10	10	10	10	10

DATE		2013.08.04 - Day 10											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	10	10	10
Control discharge water	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 25%	30	0	0	0	0	0	0	10	10	10	10	10	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	10	10	10	10	10

DATE		2013.08.05 - Day 11											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	10	10	10
Control discharge water	30	0	0	0	0	1	0	10	10	10	10	9	10
Treated discharge water 25%	30	0	0	0	0	5	0	10	10	10	10	5	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	10	10	10	10	10

DATE		2013.08.06 - Day 12											
Concentration (%)	Total number of eggs	Malformed larvae			Dead larvae			Total hatched larvae			Total live larvae		
		A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	10	10	10
Control discharge water	30	0	0	0	0	5	0	10	10	10	10	4	10
Treated discharge water 25%	30	0	0	0	0	2	0	10	10	10	10	3	10
Treated discharge water 50%	30	0	0	0	0	0	0	8	10	10	8	10	10
Treated discharge water 100%	30	0	0	0	0	0	0	10	10	10	10	10	10

C.2 Measurements of pH. oxygen and salinity

Summary of measurement of pH. oxygen and salinity (all test vessels)				
Parameter	Temperature (°c)	Dissolved oxygen (%)	Salinity (psu)	pH (unit)
Minimum	22.9	65	15.2	7.8
Maximum	25.9	100	20.7	8.2
Average	24.9	88	19.1	8.0
STD	0.8	7.7	1.3	0.1

DATE	2013.07.25 - Day 0											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	23.2	23.2	23.2	100	100	100	18.3	18.3	18.3	8.1	8.1	8.1
Control discharge water	24.6	24.6	24.6	75	75	75	19.9	19.9	19.9	7.9	7.9	7.9
Treated discharge Water 25%	24.5	24.5	24.5	90	90	90	18.8	18.8	18.8	8.0	8.0	8.0
Treated discharge water 50%	24.3	24.3	24.3	94	94	94	17.5	17.5	17.5	8.0	8.0	8.0
Treated discharge water 100%	24.2	24.2	24.2	96	96	96	15.2	15.2	15.2	8.0	8.0	8.0

DATE	2013.07.29 - Day 4 (before renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	25.9	25.8	25.8	96	97	96	18.5	18.5	18.5	8.0	7.9	8.0
Control discharge water	25.8	25.8	25.7	97	99	99	20.1	20.1	20.2	8.1	8.1	8.1
Treated discharge Water 25%	25.8	25.7	25.8	91	92	92	19.3	19.3	19.0	8.1	8.1	8.1
Treated discharge water 50%	25.7	25.7	25.7	98	96	99	17.9	17.9	17.8	8.1	8.1	8.1
Treated discharge water 100%	25.7	25.7	25.7	99	100	99	15.4	15.4	15.4	8.1	8.1	8.0

DATE	2013.07.29 - Day 4 (after renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	24.6	24.6	24.6	94	94	94	19.3	19.3	19.3	8.0	8.0	8.0
Control discharge water	24.8	24.8	24.8	77	77	77	19.9	19.9	19.9	7.9	7.9	7.9
Treated discharge Water 25%	25.0	25.0	25.0	89	89	89	19.9	19.9	19.9	8.0	8.0	8.0
Treated discharge water 50%	25.1	25.1	25.1	88	88	88	19.9	19.9	19.9	8.0	8.0	8.0
Treated discharge water 100%	25.4	25.4	25.4	92	92	92	19.9	19.9	19.9	8.1	8.1	8.1

DATE	2013.07.31 - Day 6 (before renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	25.4	25.5	25.4	80	81	82	19.5	19.4	19.3	7.9	7.9	7.9
Control discharge water	25.5	25.4	25.5	81	81	79	20.2	20.2	20.2	8.0	8.1	8.0
Treated discharge Water 25%	25.2	25.3	25.3	82	81	82	20.0	20.0	20.0	8.1	8.1	8.1
Treated discharge water 50%	25.2	25.4	25.3	83	80	80	19.9	19.9	19.8	8.1	8.1	8.1
Treated discharge water 100%	25.4	25.5	25.4	82	80	81	19.8	19.9	19.9	8.1	8.1	8.1

DATE	2013.07.31 - Day 6 (after renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	24.3	24.3	24.3	82	82	82	19.9	19.9	19.9	7.9	7.9	7.9
Control discharge water	24.6	24.6	24.6	65	65	65	19.5	19.5	19.5	7.9	7.9	7.9
Treated discharge Water 25%	24.7	24.7	24.7	76	76	76	19.0	19.0	19.0	7.9	7.9	7.9
Treated discharge water 50%	24.8	24.8	24.8	77	77	77	18.7	18.7	18.7	8.0	8.0	8.0
Treated discharge water 100%	25.2	25.2	25.2	78	78	78	17.5	17.5	17.5	8.0	8.0	8.0

DATE	2013.08.02 - Day 8 (before renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	25.8	25.7	25.7	92	92	92	20.0	19.9	19.9	8.0	7.9	7.9
Control discharge water	25.7	25.7	25.6	98	98	98	20.1	20.0	20.0	8.1	8.1	8.1
Treated discharge Water 25%	25.5	25.6	25.7	88	87	91	19.3	19.2	19.2	7.9	7.9	8.0
Treated discharge water 50%	25.6	25.7	25.7	92	94	93	19.1	19.0	19.0	8.0	8.0	8.1
Treated discharge water 100%	25.8	25.7	25.6	96	97	97	18.1	18.0	17.9	8.1	8.1	8.1

DATE	2013.08.02 - Day 8 (after renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	24.6	24.6	24.6	95	95	95	20.5	20.5	20.5	8.0	8.0	8.0
Control discharge water	23.8	23.8	23.8	79	79	79	19.7	19.7	19.7	8.1	8.1	8.1
Treated discharge Water 25%	24.4	24.4	24.4	90	90	90	18.8	18.8	18.8	8.1	8.1	8.1
Treated discharge water 50%	24.4	24.4	24.4	93	93	93	18.0	18.0	18.0	8.1	8.1	8.1
Treated discharge water 100%	24.9	24.9	24.9	95	95	95	15.8	15.8	15.8	8.1	8.1	8.1

DATE	2013.08.05 - Day 11 (before renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	25.9	25.8	25.8	90	85	84	20.5	20.6	20.7	8.0	7.9	7.8
Control discharge water	25.8	25.8	25.8	93	93	93	20.0	19.8	19.9	8.1	8.1	8.1
Treated discharge Water 25%	25.8	25.8	25.8	95	92	93	19.0	19.0	19.0	8.1	8.1	8.1
Treated discharge water 50%	25.7	25.7	25.8	92	92	93	18.0	18.1	18.2	8.1	8.1	8.1
Treated discharge water 100%	25.8	25.7	25.7	95	95	94	16.2	16.0	16.1	8.2	8.2	8.2

DATE	2013.08.05 - Day 11 (after renewal)											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	23.8	23.8	23.8	92	92	92	20.5	20.5	20.5	8.1	8.1	8.1
Control discharge water	22.9	22.9	22.9	75	75	75	19.7	19.7	19.7	8.2	8.2	8.2
Treated discharge Water 25%	23.2	23.2	23.2	85	85	85	19.5	19.5	19.5	8.2	8.2	8.2
Treated discharge water 50%	23.1	23.1	23.1	87	87	87	19.5	19.5	19.5	8.2	8.2	8.2
Treated discharge water 100%	23.2	23.2	23.2	87	87	87	19.1	19.1	19.1	8.2	8.2	8.2

DATE	2013.08.06 - Day 12											
Concentration (%)	Temp. (°C)			Oxygen (% saturation)			Salinity (PSU)			pH		
	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	25.6	25.6	25.5	82	85	83	20.4	20.4	20.4	7.9	7.9	7.9
Control discharge water	25.5	25.5	25.5	87	85	87	19.7	19.6	19.8	8.0	8.0	8.0
Treated discharge Water 250%	25.3	25.4	25.5	87	87	87	19.4	19.4	19.4	7.9	7.9	7.9
Treated discharge water 50%	25.5	25.5	25.6	88	90	90	19.2	19.3	19.3	8.0	8.0	8.0
Treated discharge water 100%	25.6	25.5	25.5	88	90	89	18.9	18.8	18.9	8.1	8.1	8.1

C.3 Validity criteria

Date	Criteria	Target Value	Fulfilled
Validity criteria	Survival of hatched larvae in the control (S)	S > 80%	YES
	Hatching Success in the control (HS)	HS > 75%	YES
	Dissolved oxygen concentration (DO)	DO > 60	YES
	Water temperature differing (T)	24 °C ± 1.5 °C	YES

C.4 Statistic

C.4.1 Survival at larval stage

Replicate	Laboratory control	Control discharge water	Treated discharge water 25%	Treated discharge water 50%	Treated discharge water 100%
A	100	100	100	100	100
B	100	40	30	100	100
C	100	100	100	100	100
n	3	3	3	3	3
Mean	100	80.0	76.7	100	100
STD	0	34.6	40.4	0	0
P-value	0.19	-	0.46	0.19	0.19
Significantly smaller than control discharge	NO	-	NO	NO	NO

C.4.2 Survival at embryo stage

Replicate	Laboratory control	Control discharge water	Treated discharge water 25%	Treated discharge water 50%	Treated discharge water 100%
A	100	100	100	80	100
B	100	100	100	100	100
C	100	100	100	100	100
n	3	3	3	3	3
Mean	100	100	100	93.3	100
STD	0	0	0	11.5	0
P-value	1.00	-	1.00	0.19	1.00
Significantly smaller than control discharge	NO	-	NO	NO	NO

C.4.3 Overall survival

Replicate	Laboratory control	Control discharge water	Treated discharge water 25%	Treated discharge water 50%	Treated discharge water 100%
A	100	100	100	80	100
B	100	40	30	100	100
C	100	100	100	100	100
n	3	3	3	3	3
Mean	100	80.0	76.7	93.3	100
STD	0	34.6	40.4	11.5	0
P-value	0.19	-	0.46	0.19	0.19
Significantly smaller than control discharge	NO	-	NO	NO	NO

C.4.4 Larval growth test

Fish No.	Laboratory control			Control discharge water			Treated discharge water 25%			Treated discharge water 50%			Treated discharge water 100%		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	3.9	3.8	3.8	3.8	3.7	3.7	3.5	3.6	3.7	3.6	3.8	3.7	3.6	3.7	3.8
2	3.8	3.9	4.0	3.8	3.7	3.8	3.7	3.7	3.7	3.6	3.8	3.6	3.6	3.7	3.8
3	3.9	3.8	3.8	3.7	3.6	3.8	3.7	3.6	3.8	3.6	3.8	3.7	3.7	3.7	3.8
4	3.8	3.8	4.0	3.8	3.6	3.8	3.7	-	3.6	3.5	3.7	3.7	3.8	3.8	3.6
5	3.9	4.0	3.8	3.8	-	3.7	3.6	-	3.6	3.7	3.7	3.7	3.7	3.8	3.8
6	4.0	3.8	3.9	3.9	-	4.0	3.8	-	3.7	3.7	3.6	3.7	3.8	3.7	3.9
7	3.9	3.9	3.8	3.7	-	3.8	3.7	-	3.6	3.7	4.0	3.7	3.6	3.8	3.7
8	4.0	3.8	3.8	3.8	-	3.8	3.6	-	3.6	3.7	3.8	3.7	3.8	3.7	3.6
9	3.9	4.0	4.0	3.7	-	3.8	3.6	-	3.8	-	3.7	3.7	3.7	3.8	3.6
10	3..8	3.6	3.8	3.7	-	3.8	3.6	-	3.6	-	3.7	3.7	3.5	3.6	3.7
n	30			24			23			28			30		
Mean	3.9			3.8			3.7			3.7			3.7		
STD	0.1			0.1			0.1			0.1			0.1		
Min	3.6			3.6			3.5			3.5			3.5		
Max	4.0			4.0			3.8			4.0			3.9		
P-value	0.00			-			0.00			0.01			0.03		
Significantly smaller than control discharge (α=0.01)	NO			-			YES			YES			NO		

APPENDIX D

Certificate of compliance, ISO 9001 certificate, accreditation and GLP authorisation

COPY

Certificate no: **DS/I093222-A**
Page 1 of 1

Lloyd's Register

Certificate of Compliance

Office: **Lloyd's Register EMEA**
Copenhagen Design Support Centre, Statutory Section
Strandvejen 104A, 2nd floor
DK-2900 Hellerup
Denmark

Date: **09 May 2012**

This certificate is issued to **DHI Ballast Water Centre, Denmark**

DHI Ballast Water Centre, Denmark

The Document(s) listed in paragraph 1 of the appendix have been examined for compliance with:

- Resolution MEPC.174(58), Annex part 2

and are found to comply from quality assurance and quality control aspects subject to the following:

1.1. It is required to maintain full and accurate log files in order to demonstrate correct quality measures

1.2. The Quality Assurance Project Plan is a project specific document and should as such be subject to review and commenting prior to each project start-up.

1.3. This design appraisal document is to be kept together with quality management plan.

1.4. Subject certificate is valid until 15 June 2015.

1. The documents listed below have been examined

Drawing No.	Rev.	Title	Status	Date
Date: 07 Sep 2011	**2.3**	**Quality Management Plan**	**B**	**09 May 2012**

2. The documents listed below have been considered together with the submitted documents in the appraisal

Drawing No.	Rev.	Title
11810704	**02**	**Quality Assurance Project Plan**

Appraisal Status Key

B Examined and found to comply with §2.2, Part 2 of the annex of IMO Resolution MEPC 174 (58)

Martin Schabert
Statutory Department
Copenhagen Design Support Centre
Surveyor to Lloyd's Register EMEA

LLOYD'S REGISTER EMEA
Lloyd's Register
M. Schabert
COPENHAGEN DESIGN SUPPORT CENTRE

A member of the Lloyd's Register Group

Form 1124 (2005.02)

DNV BUSINESS ASSURANCE

MANAGEMENT SYSTEM CERTIFICATE

Certificate No. 109333-2012-AQ-DEN-DANAK

This is to certify that

DHI Group

has been found to conform to the management system standard:

DS/EN ISO 9001:2008

This certificate is valid for the following product or service ranges:

Consulting, software, research & development and laboratory testing, analysis & products within the area of water, environment & health

Locations included in the certification will appear in the appendix.

Place and date:

Hellerup, 2014-01-14

DET NORSKE VERITAS,
BUSINESS ASSURANCE, DANMARK A/S

This certificate is valid until:

2015-01-10

The audit has been performed under the supervision of:

DANAK
SYSTEM Reg.nr. 5001

Henrik Bjørnstrup
Lead Auditor

Lars Appel
Managing Director

Lack of fulfilment of conditions as set out in the Certification Agreement may render this certificate invalid.

ACCREDITED UNIT: DET NORSKE VERITAS, BUSINESS ASSURANCE, DANMARK A/S, TUBORG PARKVEJ 8, 2., DK-2900, HELLERUP, DANMARK, TEL:+45 39 45 48 00, WWW.DNVBA.COM

DNV BUSINESS ASSURANCE

APPENDIX TO CERTIFICATE

This appendix refers to certificate no. 109333-2012-AQ-DEN-DANAK

DHI Group

Locations included in the certification are as follows:

Site Address	Scope:
Agern Allé 5 2970 Hørsholm, Denmark	Consulting, MIKE by DHI Software Development, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support, Solutions Software development, Research, Development & Innovation, Laboratory Analysis, Testing & Products
INCUBA Science Park, Gustav Wieds Vej 10 8000 Århus, Denmark	Consulting, Solutions Software Development and Research, Development & Innovation
c/o Department of Geography and Geology, University of Copenhagen, Øster Voldgade 10, DK-1350 Copenhagen K, Denmark	Consulting
Drakegatan 6, S-412 50 Göteborg, Sweden	Consulting, MIKE by DHI & MIKE by DHI Customised Software Support, Research, Development & Innovation
Södra Tullgatan 4, S-211 41 Malmö, Sweden	Consulting, MIKE by DHI & MIKE by DHI Customised Software Sales, MIKE by DHI & MIKE by DHI Customised Software Support, Research, Development & Innovation
Svartmangatan 18, S-111 29 Stockholm, Sweden	Consulting, MIKE by DHI & MIKE by DHI Customised Software Support, Research, Development & Innovation

Lack of fulfilment of conditions as set out in the Certification Agreement may render this certificate invalid.

ACCREDITED UNIT: DET NORSKE VERITAS, BUSINESS ASSURANCE, DANMARK A/S, TUBORG PARKVEJ 8, 2., DK-2900, HELLERUP, DANMARK, TEL:+45 39 45 48 00, WWW.DNVBA.COM

Honnörsgatan 16, Box 3289, S-350 53 Växjö, Sweden	Consulting, MIKE by DHI & MIKE by DHI Customised Software Sales, MIKE by DHI & MIKE by DHI Customised Software Support, Research, Development & Innovation
1 CleanTech Loop, #03-05 CleanTech One Singapore 637141, Singapore	Consulting, MIKE by DHI & MIKE by DHI Customised Software Sales MIKE by DHI & MIKE by DHI Customised Software Support, Solutions Software Development, Research, Development & Innovation, Laboratory Analysis & Testing, Health & Safety, Environment
3A01-02, Block G, Pusat Dagangan Phileo Damansara I, No. 9, Jalan 16/11, Off Jalan Damansara, 46350 Petaling Jaya, Selangor, Malaysia	Consulting, MIKE by DHI Software Sales & Solutions Software Development, Research, Development & Innovation
11th Floor, Wisma Perindustrian, Jalan Istiadat, Likas, 88400 Kota Kinabalu, Sabah, Malaysia	Consulting, MIKE by DHI Software Sales and Solutions Software Development, Research, Development & Innovation
IIIrd Floor, NSIC – STP Complex, NSIC Bhawan, Okhla Industrial Estate, New Delhi – 110020, India	Consulting, MIKE by DHI Software Sales & Support and Solutions Software Development, Research, Development & Innovation
Level 5, 67 Astor Terrace, Spring Hill Brisbane QLD 4000, Australia	Consulting, Solutions Software Development
Level 2, 12 Short Street Southport QLD 4215, Gold Coast, Australia	Consulting, MIKE by DHI & MIKE by DHI Customised Software Sales MIKE by DHI & MIKE by DHI Customised Software Support
Suite 8.01, Level 8, 50 Clarence Street, Sydney NSW 2001, Australia	Consulting
Suite 146, Equus Center 580 Hay Street, Perth WA 6000, Australia	Consulting
101 E Centre, Oaklands Rd, Albany 0752, Auckland, New Zealand	Consulting, MIKE© by DHI Software Sales & Support and Solutions Software Development
First Floor, 192 Papanui Road Merivale, Christchurch 8141, New Zealand	Consulting, MIKE© by DHI Software Sales & Support and Solutions Software Development
2/4 rue Edouard Nignon, CS 47202, 44372 Nantes Cedex 3	Consulting, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support
Waltersdorfer Straße 105, 12526 Berlin-Bohnsdorf	Consulting, MIKE© by DHI Software Development, Sales & Support
Comeniusstraße 109, 01309 Dresden	Consulting, Solutions Software development

Lack of fulfilment of conditions as set out in the Certification Agreement may render this certificate invalid.

ACCREDITED UNIT: DET NORSKE VERITAS, BUSINESS ASSURANCE, DANMARK A/S, TUBORG PARKVEJ 8, 2., DK-2900, HELLERUP, DANMARK, TEL:+45 39 45 48 00, WWW.DNVBA.COM

Max-Planck-Straße 6, 28857 Syke, Germany	Consulting, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support
Volmerstraße 8, 12489 Berlin, Germany	Consulting MIKE by DHI Software Development, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support, Solutions Software development
Via Pomba 23, 10123 Torino, Italy	Consulting, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support
Via Operai, 40/19, 16149 Genova, Italy	Consulting, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support
Gaustadalléen 21, 0349 Oslo, Norway	Consulting, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support
Abels gate 5, 7030 Trondheim, Norway	Consulting, MIKE by DHI & MIKE by DHI Customised software sales, MIKE by DHI & MIKE by DHI Customised software support

Place and date:

Hellerup, 2014-01-14

DET NORSKE VERITAS,
BUSINESS ASSURANCE, DANMARK A/S

This certificate is valid until:

2015-01-10

The audit has been performed under the supervision of:

DANAK
SYSTEM Reg.nr. 5001

Henrik Bjørnstrup
Lead Auditor

Lars Appel
Managing Director

Lack of fulfilment of conditions as set out in the Certification Agreement may render this certificate invalid.

ACCREDITED UNIT: DET NORSKE VERITAS, BUSINESS ASSURANCE, DANMARK A/S, TUBORG PARKVEJ 8, 2., DK-2900, HELLERUP, DANMARK, TEL:+45 39 45 48 00, WWW.DNVBA.COM

Accreditation to testing

COPY

DANAK

Company:	**DHI**
	Agern Allé 5 **DK-2970 Hørsholm**
Registration number:	**26**
Valid:	**24-10-2012 to 31-07-2015**

Scope:

Testing

Product

- **Biological items**
- **Chemicals and chemical products**
- **Construction products**
- **Environmental samples**

Test Type

- **Biological and biochemical testing**
- **Chemical testing**
- **Microbiological testing**
- **Ionising radiation and radiochemistry**
- **Sampling**

Testing is performed according to the current list of test methods approved by DANAK.

The company complies with the criteria in EN ISO/IEC 17025:2005 – General requirements for the competence of testing and calibration laboratories and demonstrates technical competence for the defined scope and the operation of a quality management system (refer joint ISO-ILAC-IAF Communiqué dated January 2009, www.danak.dk).

Issued the 24 October 2012

f. **Jesper Høy** / **Kirsten Jebjerg Andersen**

In case of any disputes, the Document in Danish language shall have priority.

Den Danske Akkrediterings- og Metrologifond

COPY

DANAK

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Laboratory inspection and study audits for compliance with the OECD Principles for Good Laboratory Practice were carried out at

Laboratory: DHI

on

Dates: 21st and 22nd October 2011

The laboratory inspection and study audits have been carried out in accordance with the regulation settled in Order No. 906 of 14th September 2009 from the Danish Ministry of Environment. The laboratory has been monitored for GLP Compliance within the following scope:

Type of products:

- *Industrial chemicals*
- *Pesticides*
- *Biocides*

Type of tests:

- *Environmental toxicity studies on aquatic and terrestrial organisms.*
- *Studies of behaviour in water, soil and air, bioaccumulation*

The laboratory was found to be operating in compliance with the OECD Principles of Good Laboratory Practice.

Date: 08 August 2012

Jesper Høy
Managing director, DANAK

Kirsten Jebjerg Andersen
GLP inspector, DANAK

Den Danske Akkrediterings- og Metrologifond